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Microvirga makkahensis sp. nov., and *Microvirga arabica* sp. nov., isolated from sandy arid soil

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Abstract The taxonomic positions of two Gram-negative strains, SV1470^T and SV2184P^T, isolated from arid soil samples, were determined using a polyphasic approach. Analysis of the 16S rRNA gene and the concatenated sequences of three housekeeping gene loci (*dna*K, *rpo*B and *gyr*B) confirmed that the strains belong to the genus *Microvirga*. Strain SV1470^T was found to be closely related to *Microvirga vignae* BR3299^T (98.8 %), *Microvirga flocculans* TFB^T (98.3 %) and *Microvirga lupini* Lut6^T (98.2 %), whilst similarity to other type strains of the genus ranged from 97.8 to 96.3 %; strain SV2184P^T was found to be closely related to *Microvirga aerilata* 5420S-16^T (98.0 %), *Microvirga zambiensis* WSM3693^T (97.8 %) and *M. flocculans* ATCC BAA-817^T (97.4 %), whilst similarity to other type strains of the genus ranged from 97.2 to 95.9 %. The G + C content of the genomic DNA was determined to be 61.5 mol % for strain SV1470^T and 62.1 mol % for strain SV2184P^T. Both strains were found to have the same quinone system, with Q-10 as the major ubiquinone. The polar lipid profile of strain SV1470^T was found to consist of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and one unidentified aminolipid, while that of strain SV2184P^T consisted of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, one unidentified aminolipid, one unidentified aminophospholipid and two unidentified phospholipids. DNA–DNA relatedness studies showed that the two strains belong to different genomic species. The strains were also distinguished using a combination

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of phenotypic properties. Based on the genotypic and phenotypic data, the novel species *Microvirga makkahensis* sp. nov. (type strain SV1470^T = DSM 25394^T = KCTC 23863^T = NRRL-B 24875^T) and *Microvirga arabica* sp. nov. (type strain SV2184P^T = DSM 25393^T = KCTC 23864^T = NRRL-B 24874^T) are proposed.

Keywords *Proteobacteria* · *Alphaproteobacteria* · *Microvirga arabica* · *Microvirga makkahensis* · Polyphasic taxonomy

Introduction

The genus *Microvirga* was described by Kanso and Patel (2003) and emended descriptions have been given by Zhang et al. (2009), Weon et al. (2010) and Ardley et al. (2012). The genus *Microvirga* belongs to the α-2 subclass of the *Proteobacteria* and its members are strictly aerobic, Gram-stain negative, motile, non-sporulating short rods that produce pink-pigmented colonies. The genus *Microvirga* currently contains nine validly named species which have been isolated from a subsurface geothermal aquifer (*Microvirga subterranea*; Kanso and Patel 2003), environmental samples (*Microvirga guangxiensis*; Zhang et al. 2009), air samples (*Microvirga aerophila* and *Microvirga aerilata*; Takeda et al. 2004 and Weon et al. 2010), a hot spring (*Microvirga flocculans*; Takeda et al. 2004 and Weon et al. 2010), legume nodules(*Microvirga lupini, Microvirga lotononidis* and *Microvirga zambiensis*; Ardley et al. 2012) and cowpea nodules (*Microvirga vignae*; Radl et al. 2014).

Strains SV1470^T and SV2184P^T were isolated from a soil sample collected in front of the Hira Cave, Makkah (Mekka), Saudi Arabia. The aim of this study was to determine the taxonomic position of the isolates using a polyphasic approach. Based on genotypic and phenotypic data, we conclude these strains represent two novel species in the genus *Microvirga*.

Materials and methods

Isolation and maintenance of the organisms

Strains SV1470^T and SV2184P^T were isolated on Stevenson's medium No 1 and No 2 (Tan et al. 2006), respectively, supplemented with cycloheximide (50 μg ml^{-1}), neomycin sulphate (4 μg ml^{-1}) and nystatin (50 μg ml^{-1}), after incubation for 21 days at 28 °C, following inoculation with a suspension of a soil sample. The soil samples were collected in front of the cave of Hira, Makkah, Saudi Arabia (GPS coordinates for the sampling site are 21°27′25.69″N and 39°51′33.04″E). The organisms were maintained on glucose-yeast extract-malt extract agar slopes (GYM; DSMZ catalog No.65; http://www.dsmz.de/microorganisms/medium/pdf/DSMZ Medium 65.pdf) at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C.

The type strains *M. aerilata* KACC 12744^T, *M. flocculans* KCTC 12101^T, *M. zambiensis* HAMBI 3238^T, *M. lupini* HAMBI 3236^T, *M. lotononidis* HAMBI 3237^T and *M. vignae* BR3299^T were obtained from the culture collections indicated by the respective type strain codes. Reference strains were maintained on GYM and as glycerol suspensions (20 %, v/v) at −20 °C.

Morphological, cultural and physiological characteristics

Phenotypic characteristics of strains SV1470^T and SV2184P^T were examined using several standard methods. The reference type strains were included for comparison in all tests. Motilities of strains SV1470^T and SV2184P^T were tested by using the media and methods established by Greene et al. (1951). For transmission electron microscope (TEM) images, a sample of bacteria was deposited on formvar-carbon coated grids, washed with deionized water, negatively stained with 2 % uranyl acetate and examined using a JEOL JEM-1400 transmission electron microscope at 80 kV. Growth was tested at different temperatures (4, 10, 20, 28, 37, 45, 50 and 55 °C), pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0) and in the presence of sodium chloride (1, 2, 3, 4 and 5 %; w/v) using GYM agar as the basal medium. Reduction of nitrate, hydrolysis of aesculin, arbutin, allantoin and urea were examined as described by Gordon et al. (1974). Established methods were used to determine whether the strains degraded Tween 20 and 80 (Nash and Krent 1991); the remaining degradation tests were carried out using the media and methods described by Williams et al. (1983). Carbon source utilisation was tested using carbon source utilisation (ISP 9) medium

(Shirling and Gottlieb 1966) supplemented with a final concentration of 1 % (w/v) of the tested carbon sources. Nitrogen source utilisation was examined using the basal medium recommended by Williams et al. (1983) supplemented with a final concentration of 0.1 % (w/v) of the tested nitrogen sources. Tests in the commercial system API-CORYNE and API-ZYM (Biomerieux) were performed according to the manufacturer's instructions.

Chemotaxonomic characterisation

Chemotaxonomic analyses were carried out to support the phylogenetic affiliation of strains SV1470^T and SV2184P^T to the genus *Microvirga*. The strains were grown in ISP 2 broth under aerobic conditions in flasks on rotary shaker at 160 r.p.m. and 28 °C for 14 days. Biomass was harvested by centrifugation, washed twice in distilled water and re-centrifuged and freeze-dried. Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890 N instrument, fitted with an autosampler and a 6783 injector, according to the standard protocol of the Sherlock Microbial identification (MIDI) system (Sasser 1990; Kämpfer and Kroppenstedt 1996); the fatty acid methyl ester peaks were quantified using the TSBA 5.0 database. Polar lipid and respiratory ubiquinone analyses were carried out by the Identification Service of the Leibniz Institute DSMZ, Braunschweig, Germany. Polar lipid analysis was carried out according to the protocol of Minnikin et al. (1984). Respiratory quinones were extracted from 100 mg of freeze dried cells based on the two stage method described by Tindall (1990a, b). Respiratory quinones were separated into their different classes (menaquinones and ubiquinones) by thin layer chromatography on silica gel (Macherey–Nagel Art. NO. 805 023), using hexan: *tert*-butylmethylether (9:1 v/v) as solvent. UV absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on a LDC Analytical (Thermo Separation Products) HPLC fitted with a reverse phase column (Macherey–Nagel, 2 mm × 125 mm, 3 µm, RP18) using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

The DNA G + C content of the isolate was determined following the procedure of Gonzalez and Saiz-Jimenez (2005).

*DNA preparation, amplification and determination of 16S rRNA, dna*K, *rpo*B *and gyr*B *gene sequence*

Genomic DNA was isolated from strains using the Genomic DNA Mini Kit (Invitrogen) following the instructions of the manufacturer. Extracted DNA was used as a template for the amplification of 16S rRNA, *dna*K, *rpo*B and *gyr*B genes. Primers and annealing temperatures are described in Supplementary Table S1.

The almost complete 16S rRNA gene sequences of strains SV1470^T and SV2184P^T (1435 and 1434 bp) were determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al. 2012). Multiple alignments with sequences from closely related species were performed by using the program CLUSTAL W in MEGA version 6.0 (Tamura et al. 2013). Phylogenetic trees were constructed with the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum parsimony (Kluge and Farris 1969) algorithms in MEGA 6.0 (Tamura et al. 2013). Evolutionary distances were calculated using model of Jukes and Cantor (1969). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Phylogenetic relationships of the strains were confirmed using sequences for three individual housekeeping genes (*dna*K, *rpo*B and *gyr*B). The sequences of each locus were aligned using MEGA 6.0 software (Tamura et al. 2013) and trimmed manually at the same position before being used for further analysis and deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Accession numbers for the genes used in generating the concatenated-sequence tree are listed in Supplementary Table S2. The sequences data were exported as a concatenated three-gene alignment for subsequence analysis using model of Jukes and Cantor (1969), using neighbour-joining (Saitou and Nei 1987) algorithm with 1000 bootstrap replication in MEGA 6.0 (Tamura et al. 2013).

DNA–DNA hybridization

DNA–DNA hybridization experiments were performed with strain SV1470^T and the related type strains *M. vignae* BR3299^T, *M. flocculans* KCTC 12101^T and *M.*

lupini HAMBI 3236^T; with strain SV2184P^T and *M. aerilata* KACC 12744^T and *M. zambiensis* HAMBI 3238^T; and with strain SV1470^T and SV2184P^T. DNA–DNA hybridizations were performed by the Identification Service of the Leibniz Institute DSMZ, Braunschweig, Germany. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) [incorporating the modifications described by Huss et al. (1983)] using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in situ temperature probe (Varian).

Results and discussion

Colonies of strains SV1470^T and SV2184P^T were observed to be pink, circular and convex with entire smooth edges and shiny surfaces. Rod-shaped cells with round ends were observed to occur singly and as pairs and to be motile with a single polar flagellum (Figs. 1, 2). Strains SV1470^T and SV2184P^T could be differentiated from each other and phylogenetically related *Microvirga* species based on physiological and biochemical properties such as degradation of Tween 20 and utilisation of adonitol, D-sorbitol and alpha-iso-leucine as carbon and nitrogen sources. Detailed phenotypic characteristics are given in the Table 1 and species descriptions.

The major cellular fatty acids were identified as $C_{18:1}$ w7c (52.0 %) and $C_{19:0}$ *cyclo* w8c (18.8 %) for strain SV1470^T, and $C_{18:1}$ w7c (75.8 %) for strain SV2184P^T (Table 2). Q-10 was identified as the major ubiquinone for both strains SV1470^T and SV2184P^T. The polar lipid profile of strain SV1470^T was found to consist of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and one unidentified aminolipid, while that of strain SV2184P^T consisted of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, one unidentified aminolipid, one unidentified aminophospholipid and two unidentified phospholipids (Supplementary Fig. S1, S2).

Preliminary 16S rRNA gene sequence comparisons in GenBank indicated that two novel isolates are related to members of the genus *Microvirga*. 16S rRNA gene sequence analysis indicated that they shared 97.8 % sequence similarity (35 nt differences at 1431 locations) with each other. Sequence similarity calculation after neighbour-joining analysis (Fig. 3) indicated that close relatives of strain SV2184P^T are *M. aerilata* 5420S-16^T (98.04 %; 28 nt differences in 1431), *M. zambiensis* WSM3693^T (97.83 %; 31 nt differences in 1431) and *M. flocculans* ATCC BAA-817^T (97.41 %; 37 nt differences in 1431). Lower

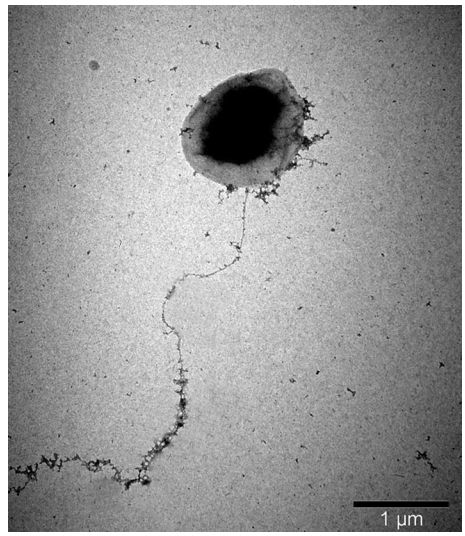

Fig. 1 Transmission electron micrograph (TEM) showing a single polar flagellum of strain SV1470^T. *Bar* 1 µm

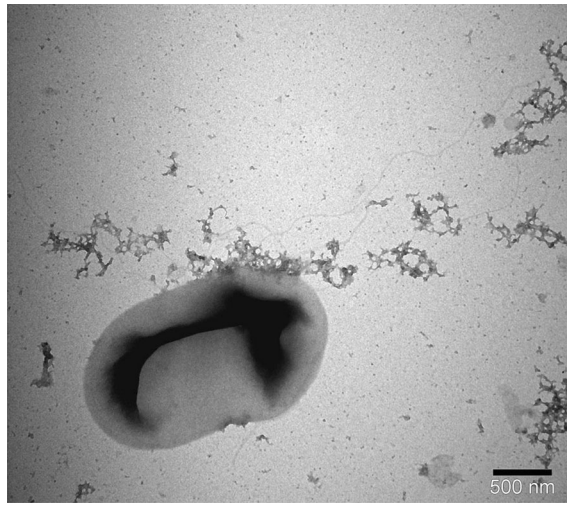

Fig. 2 Transmission electron micrograph (TEM) showing a single polar flagellum of strain SV2184P^T. *Bar* 500 nm

Table 1 Phenotypic properties of strains SV1470^T, SV2184P^T and closely related type species

	1	2	3	4	5	6	7	8
Cell Morphology								
Cell length (μm)	1.0–1.6	2.0[a]	0.6–1.3	1.4–2.5	0.7–1.8	0.6–1.6	1.0–2.2[a]	1.0–2.2[a]
Cell width (μm)	0.8–1.3	0.87[a]	0.4–0.7	1.2–1.8	0.5–0.7	0.7–0.9	0.4–0.5[a]	0.4–0.5[a]
Motility	Motile	Motile	Non-Motile	Motile	Non-motile	Motile	Non-motile	Motile
Biochemical Tests								
Nitrate reduction	−	−	−	−	−	−	−	+
Urea hydrolysis	−	−	−	−	−	−	+	+
Growth at/on								
45 °C	+	−	+	−	−	−	−	+
1 % (w/v) NaCl	+	+	−	+	−	−	+	+
2 % (w/v) NaCl	+	−	−	−	−	−	−	+
Degradation of								
Tween 20	+	−	−	−	−	−	−	−
Tween 80	−	−	−	+	−	−	−	−
Utilization as carbon source (1.0 %, w/v)								
Adonitol	+	+	−	+	−	−	+	−
D-sorbitol	+	−	−	+	−	−	−	−
Dextrin	−	−	−	+	+	+	−	+
L-arabinose	+	+	+	+	+	−	+	+
Succinic acid (0.1 %)	+	−	−	+	−	+	+	+
Utilization as nitrogen source (0.1 %, w/v)								
Alpha-iso-leucine	+	−	+	+	+	−	−	+
L-serine	−	−	−	+	−	−	−	−
L-threonine	−	−	+	+	+	+	−	−
API-ZYM								
Alkaline phosphatase	+	−	+	+	+	+	−	−
Esterase	−	−	+	+	−	−	−	−
Esterase lipase	+	−	+	+	+	+	−	+
Leucine arylamidase	−	+	−	+	−	+	+	+
Valine arylamidase	−	+	−	+	−	+	−	+
Cystine arylamidase	−	+	+	+	−	+	+	+
Trypsin	−	−	+	−	−	+	−	−
α-chymotrypsin	−	−	−	−	−	−	+	−
Acid phosphatase	−	−	+	−	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	−	+	−	−	+	−	+
API-CORYNE								
Gelatine	+	−	+	−	−	−	−	+
Mannitol	−	+	+	−	−	−	−	+
Glucose	+	+	+	+	+	+	−	+
Ribose	+	−	+	+	+	+	−	+
Xylose	+	−	+	+	+	+	−	+
Maltose	−	+	+	−	−	−	−	+
Lactose	+	−	−	+	−	−	−	+
Sucrose	−	−	+	−	−	−	−	+

Table 1 continued

	1	2	3	4	5	6	7	8
Catalase	+	+	+	–	–	+	–	–

All strains are positive for D-cellobiose but negative for lipase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and glycogen

Strains: *1* strain SV1470^T, *2 M. vignae* BR3299^T, *3 M. flocculans* KCTC 12101^T, *4* strain SV2184P^T, *5 M. aerilata* KACC 12744^T, *6 M. zambiensis* HAMBI 3238^T, *7 M. lupini* HAMBI 3236^T, *8 M. lotononidis* HAMBI 3237^T.

[a] Data for cell morphology taken from Radl et al. (2014) and Ardley et al. (2012)

sequence similarities (<97.2 %) were found with other established species of the genus *Microvirga*. The relationship between strain SV2184P^T and its closest neighbours, *M. aerilata* 5420S-16^T, *M. zambiensis* WSM3693^T and *M. flocculans* ATCC BAA-817^T was supported by the maximum-likelihood and maximum-parsimony algorithm (Supplementary Fig. S3, S4). In contrast, strain SV1470^T and the type strain of *M. vignae* BR3299^T formed a phyletic branch that was supported by all of three-making algorithms but not by a high bootstrap value; the two organisms shared 98.82 % 16S rRNA gene sequence similarity, a value corresponding to 16 nt differences at 1396 locations. Sequence similarities with *M. flocculans* ATCC BAA-817^T, *M. lupini* Lut6^T, *M. subterranea* DSM 14364^T, *M. aerilata* 5420S-16^T, *M. zambiensis* WSM3693^T, *M. lotononidis* WSM 3557^T, *M. guangxiensis* 25B^T and *M. aerophila* 5420S-12^T were 98.26 % (25 nt differences in 1435), 98.19 % (26 nt differences in 1435), 97.84 % (31 nt differences in 1435), 97.77 % (32 nt differences in 1435), 97.56 % (35 nt differences in 1433), 97.42 % (37 nt differences in 1435), 97.06 % (41 nt differences in 1396) and 96.31 % (53 nt differences in 1435), respectively. Sequence similarities are summarised in Table 3.

The concatenated sequences of three protein-coding loci (*dna*K, *rpo*B and *gyr*B) contained 2000 nt for each strain. Sequence similarities for the individual genes are listed in Table 3. The neighbour-joining tree based on the three concatenated genes had a similar topology to the 16S rRNA gene tree and strains SV1470^T and SV2184P^T could be clearly differentiated from the type strains of the genus *Microvirga* with very high bootstrap values (Fig. 4).

The taxonomic integrity of the test strains was supported by DNA relatedness data. Strain SV1470^T showed DNA relatedness values 37.0 % to *M. vignae* BR3299^T, 23.0 % to *M. flocculans* KCTC 12101^T and 31.1 % to *M. lupini* HAMBI 3236^T while strain SV2184P^T showed DNA relatedness values 33.4 % to *M. aerilata* KACC 12744^T and 34.3 % to *M. zambiensis* HAMBI 3238^T. DNA–DNA hybridization data confirmed that strains SV1470^T and SV2184P^T represent two separate species with low hybridization values (31.3 %) to each other.

Based on the genotypic and phenotypic data presented here, it is concluded that strains SV1470^T and SV2184P^T represent novel species of the genus *Microvirga*, for which the names *Microvirga makkahensis* sp. nov. (type strain SV1470^T = DSM 25394^T = KCTC 23863^T = NRRL-B 24875^T) and *Microvirga arabica* sp. nov. (type strain SV2184P^T = DSM 25393^T = KCTC 23864^T = NRRL-B 24874^T) are proposed.

Description of *Microvirga makkahensis* sp. nov

Microvirga makkahensis (mak.kah.en'sis. N.L. fem.adj. *makkahensis*, from Makkah, Saudi Arabia, source of the organism).

Cells are aerobic, Gram-stain negative, motile, asporogenous short rods. Grows well on modified Bennett's, Czapek's, nutrient, ISP 2, YMA medium, Rauff medium and R2A medium. Growth occurs at 20–45 °C (optimum 28 °C), 0–2 % NaCl (optimum 0 % NaCl) and at pH 6.0–9.0 (optimal pH 7.0). Aesculin, arbutin, allantoin and urea hydrolysis, and nitrate reduction are negative. Degrades Tween 20 and gelatin but not elastin, starch, Tween 80, guanine, xanthine and xylan. Adonitol, D-cellobiose, D-galactose, D-sorbitol, glucose, D-mannose, inulin, L-arabinose, lactose, rhamnose, succinic acid, ribose and xylose are utilised as sole carbon sources but not α-methyl D-glycoside, D-melezitose, mannitol, sucrose, dextrin and maltose. L-alanine, L-arginine, L-methionine, L-proline, alpha-iso-leucine, L-cysteine and glycine are utilised as

Table 2 Fatty acids profiles of SV1470^T, SV2184P^T and closely related type species

Fatty acids	1	2[a]	3	4	5	6
Saturated						
C $_{14:0}$	1.9	0.5	1.6	1.4	0.6	–
C $_{16:0}$	9.0	8.2	7.5	6.8	6.3	6.4
C $_{17:0}$	–	–	–	0.7	–	–
C $_{18:0}$	4.5	5.4	2.5	7.0	8.6	12.9
Unsaturated						
C $_{16:1}$ *cis* 9	4.8		1.7	4.4	2.5	1.8
C $_{20:1}$ *trans* 11	–		–	–	–	1.1
Branched						
iso-C $_{16:1}$ *cis* 9	–		1.7	–	–	–
anteiso-C $_{15:0}$	–		4.6	–	–	–
anteiso-C $_{15:0}$ 2OH	–		0.1	–	–	–
C $_{15:0}$ 3-OH	–		–	–	–	0.8
C $_{18:0}$ 3-OH	2.7	1.9	1.8	1.0	1.2	1.6
$C_{18:1}$ w7c	52.0	71.9	45.2	75.8	74.1	72.2
C $_{17:0}$ *cyclo*	2.8		3.8	–	–	–
C $_{19:0}$ *cyclo* w8c	18.8	5.9	26.6	–	1.6	2.4
Unknown 14.503	0.4		0.4	–	–	–
Unknown 19.368	0.5		1.4	1.1	–	–
Unknown 19.735	0.2		0.4	–	–	–
Summed feature 2	–	2.6	–	–	–	–
Summed feature 3	–	3.6	–	–	–	–
Summed feature 5	1.8		0.7	1.8	–	–
Summed feature 9	–		–	–	–	0.7

Strains: *1* SV1470^T, *1 M. vignae* BR3299^T, *3 M. flocculans* KCTC 12101^T, *4* SV2184P^T, *5 M. aerilata* KACC 12744^T, *6 M. zambiensis* HAMBI 3238^T

Summed feature 2 comprises $C_{16:1}$ *iso* I/$C_{14:0}$3-OH/unknown 10.938

Summed feature 3 comprises $C_{16:1}$w7c/$C_{15:0}$ *iso* 2-OH

Summed feature 5 comprises $C_{17:1}$ *iso* I/ANTEI B

Summed feature 9 comprises unknown 18.858/.846/19cy

[a] Data taken from Radl et al. (2014)

sole nitrogen sources. Does not utilise D-L phenylalanine, L-serine, L-threonine, L-hydroxyproline and L-valine as sole nitrogen sources. Positive for naphthol-AS-BI-phosphohydrolase, alkaline phosphatase, esterase-lipase, pyrazinamidase, pyrrolidonyl arylamidase and catalase; negative for acid phosphatase, leucine arylamidase, trypsin, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, esterase, lipase, α-fucosidase, α-mannosidase, β-galactosidase, β-glucuronidase, glycogen and valine arylamidase. The major isoprenoid quinone is Q-10. The major fatty acids are $C_{18:1}$ w7c and $C_{19:0}$ *cyclo* w8c. The DNA G + C content of the type strain is 61.5 %.

The type strain, SV1470^T (= DSM 25394^T - = KCTC 23863^T = NRRL-B 24875^T), was isolated from a soil sample collected in front of the Hira Cave, Mekkah, Saudi Arabia. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and partial sequences of *dna*K, *rpo*B and *gyr*B genes of strain SV1470^T are JN989300, KT832846, KT832842 and KT832844, respectively.

Description of *Microvirga arabica* sp. nov

Microvirga arabica (a.ra'bi.ca. L. fem. adj. arabica, Arabic, Arabian).

Cells are aerobic, Gram-stain negative, motile, asporogenous short rods. Grows well on modified Bennett's, Czapek's, nutrient, ISP 2, YMA medium, Rauff medium and R2A medium. Growth occurs at 20–37 °C (optimum 28–30 °C), 0–1 % NaCl (optimum 0 % NaCl) and at pH 6.0–9.0 (optimal pH 7.0). Aesculin and arbutin hydrolysis are positive but not allantoin, urea hydrolysis and nitrate reduction. Elastin, starch and Tween 80 are degraded but not guanine, Tween 20, gelatin, xanthine and xylan. Alpha-methyl-D-glycoside, adonitol, D-cellobiose, D-galactose, D-sorbitol, glucose, D-mannose, D-melezitose, inulin, L-arabinose, lactose, dextrin, ribose, xylose, succinic acid and rhamnose are utilised but not D-mannitol, maltose, sucrose as sole carbon sources. D-L-phenylalanine, L-alanine, L-arginine, L-methionine, L-proline, L-serine, L-threonine, alpha-isoleucine, L-cysteine, glycine, L-valine are utilised as sole nitrogen sources but not L-hydroxyproline. Positive for leucine arylamidase, alkaline phosphatase, esterase-lipase, cystine arylamidase, esterase, valine arylamidase, and negative for acid phosphatase, naphthol-AS-BI-phosphohydrolase, trypsin, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-chymotrypsin, lipase, α-fucosidase, α-mannosidase, β-galactosidase, glycogen, β-glucuronidase and catalase. The major isoprenoid quinone is Q-10. The major fatty acid is $C_{18:1}$ w7c. The DNA G + C content of the type strain is 62.1 %.

The type strain, SV2184P^T (= DSM 25393^T = KCTC 23864^T = NRRL-B 24874^T) was isolated from

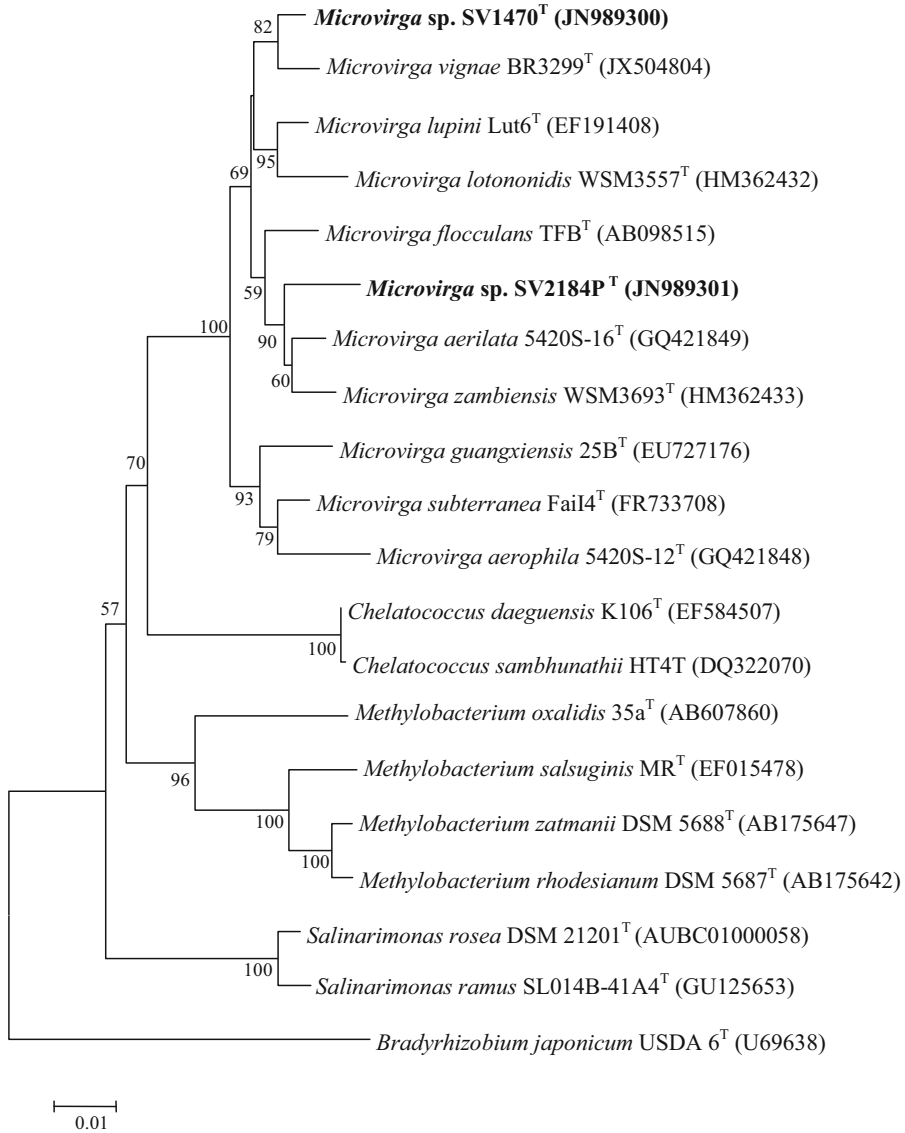

Fig. 3 Neighbour-joining phylogenetic tree based on a comparative analysis of 16S rRNA gene sequences, showing the relationships between the novel *Microvirga* strains (in *bold*) and closely related species. Numbers at nodes indicate bootstrap values (expressed as percentages of 1000 replications); only values ≥50 % are shown. GenBank accession numbers are given in parentheses. *Bradyrhizobium japonicum* USDA 6^T was used as an outgroup. *Bar* 0.01 substitutions per site

a soil sample collected in front of the Hira Cave, Mekkah, Saudi Arabia. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and partial sequences of *dna*K, *rpo*B and *gyr*B genes of strain SV2184P^T are JN989301, KT832847, KT832843 and KT832845, respectively.

Table 3 Sequence similarities (%) for the 16S rRNA, *dna*K, *rpo*B and *gyr*B genes between SV1470^T, SV2184P^T and closely related type species

Strain	Similarity (%) with strain SV1470^T				Similarity (%) with strain SV2184P^T			
	16S rRNA	*dna*K	*rpo*B	*gyr*B	16S rRNA	*dna*K	*rpo*B	*gyr*B
SV1470^T	–	–	–	–	97.80	93.54	80.88	88.85
SV2184P^T	97.80	93.54	80.88	88.85	–	–	–	–
Microvirga vignae BR3299^T	98.82	96.49	89.10	92.50	97.06	93.25	81.94	88.58
Microvirga flocculans TFB^T	98.26	94.37	80.28	92.00	97.41	94.21	89.61	87.93
Microvirga lupini Lut6^T	98.19	93.97	82.27	92.31	97.20	94.08	91.84	87.46
Microvirga subterranea FaiI4^T	97.84	92.49	83.86	90.92	96.37	91.66	85.71	89.47
Microvirga zambiensis WSM3693^T	97.56	93.83	80.88	87.69	97.83	93.81	89.24	92.26
Microvirga lotononidis WSM3557^T	97.42	93.43	82.87	91.54	96.65	93.14	92.39	90.25

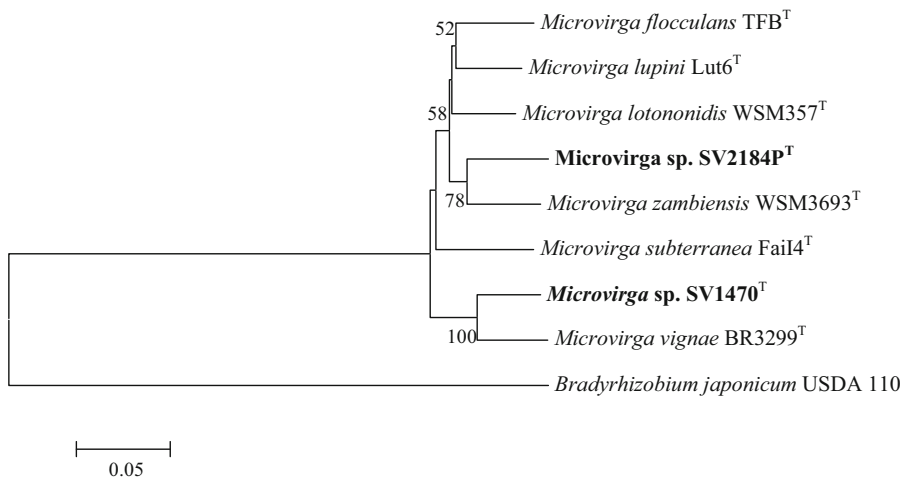

Fig. 4 Neighbour-joining phylogenetic tree based on concatenated sequences of *dna*K-*rpo*B-*gyr*B, showing the relationships between the novel *Microvirga* strains (in *bold*) and closely related species. Numbers at nodes indicate bootstrap values (expressed as percentages of 1000 replications); only values ≥50 % are shown. *Bradyrhizobium japonicum* USDA 110 was used as an outgroup. *Bar* 0.05 substitutions per site

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